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Response of *Sclerotinia sclerotiorum* against different fungicides, plant extract and bio-control agents *in vitro*

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Stem rot of *Brassica* caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, having over 500 host plants is distributed all over the world. Cultivation of mustard is becoming a threat due to the frequent occurrence of stem rot year after year in the eastern part of India including West Bengal which escalates the losses in terms of quality and quantity. Thus a study was undertaken to evaluate the efficacy of different plant extracts, fungicides and bio-agents on mycelial growth of *Sclerotinia sclerotiorum* the inciting agent of stem rot disease under *in vitro* condition by using poisoned food and dual culture techniques. The experiment was conducted at Plant pathological laboratory, Pulses and oilseeds research station, Berhampore, Murshidabad, W.B., during 2017-18. The response of fungicides indicated that carbendazim, difenconazole, and thiophenate methyl were able to inhibit the mycelial growth completely (100%) at 50 ppm concentration. Captan+ Hexaconazole inhibited 72.59 % and 100 % at 50 ppm, and 100 ppm respectively. Mancozeb was found to be less effective in inhibiting the mycelial growth of *S. sclerotiorum* and exhibited 36.29 %, 48.88% and 71.48 % growth inhibition with 50ppm, 100 ppm, and 150 ppm respectively. The extract of garlic was found most effective against *S. sclerotiorum* and a range of 53.33 % to 87.77 % growth inhibition was observed with 5-15% concentration followed by Ginger extract 42.96 to 72.59 percent with concentration. Extract of onion inhibited only 5.92 %,11.11 % and 13.11 % at 5,10 and 15% concentration which was found to be least effective. In case bio-control agents maximum 74.16 % mycelial growth inhibition of fungus was recorded with *Pseudomonas fluorescens* followed by 61.92% in *T. harzianum,* and minimum mycelial growth inhibition was recorded in *Bacillus subtilis* (42.49 %).

Key words: *Sclerotinia sclerotiorum,* plant extract, fungicide, bio-control, *Sclerotinia* stem rot, mycelia growth.

INTRODUCTION

Stem rot of Indian mustard caused by *Sclerotinia sclerotiourum* as one of the major challenges for profitable cultivation by the farming community. This disease has recently become important in India and elsewhere due to its high incidence which caused severe yield losses leading to discouragement among the growers of the crop. It has recently emerged as a serious problem for many parts of country (Kumar and Thakur, 2000). Sclerotinia rot has become an economically important yield reducing factor especially in raya (*Brassica juncea*) and is causing 40-80 percent losses in yield (Mehta *et. al*., 2010). In Uttar Pradesh it causes loss as high as 72 % (Chauhan *et al.,* 1992). The maximum Sclerotinia rot incidence recorded in the field of mustard growers of Rajasthan was 90 per cent, (NCIPM Newsletter, 2010). *Sclerotinia* stem rot of mustard caused by *Sclerotinia sclerotiorum* (Lib) de Bary has also been reported as important disease in West Bengal next to *Alternaria* blight. This disease is locally called as “Rai er Kandopocha” disease in West Bengal. Earlier, *sclerotinia* stem rot was considered as a minor disease in India but in the recent past it has become a threat due to its wide dispersion and destructive nature. *Sclerotinia sclerotiorum* belong to phylum Ascomycota, class Discomycetes, order Heliotiales and family Sclerotiniaceae. This species produces inoperculate asci from brownish sitipiate apothecia that arise from sclerotial stromata within or associated with a host plant (Whetzel, 1945). Significant increase in the sclerotial population in the soil might be due to

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monocropping and cultivation of rapeseeds-mustard under irrigated conditions which has made Sclerotinia rot very serious disease in Rajasthan, Haryana, Punjab, Assam, West Bengal, Madhya Pradesh, Uttar Pradesh and Bihar(Sharma *et al.*2015). Even this pathogen was reported in potato field from Hooghly district at Baikunthapur block by (Dutta *et al.,* 2009). As the pathogen is soil born hence it is difficult to manage and also have wide host rangefor which the present study was undertaken to formulate the effective strategies to manage this disease problem under *in vitro* evaluation of fungicides, plant extracts and bio-control agents against the pathogen.

MATERIALS AND METHODS

Evaluation of fungicides under in-vitro condition

The efficacy of the nine fungicides viz. mancozeb, carbendazim, carbendazim+ mancozeb, cymoxanil + mancozeb, difenconazole, metalaxly + mancozeb, captan + hexaconazole, thiophanate methyl, and chlorothalonil on the growth of *S. sclerotiorum* were tested under in vitro conditions by using poison food technique (Grover and Moore, 1962).Each fungicides stock solution was prepared i.e. 50,100,150 ppm by measured quantity of fungicides in a measured volume of sterilized water by following standard methodology. Potato dextrose agar (PDA) medium was prepared and sterilized at 15 lb pressure for 20 minutes. An equal volume of chemical solution and PDA was mixed in a sterilized conical flask and poured aseptically in the Petri plates. 5mm disc of the fungus from 7 day old young culture of *S.sclerotiorum* plate taken with the help of sterilized crock borrower, and placed it on centrally at each Petri plates soon after solidification of medium. Inoculated plates are incubated at 21± 1^0C temperature. Suitable control was maintained for each fungicide. The experiment was statistically carried out by using CRD (Completely randomized block design). Per cent over the control was calculated by the following formula suggested by Bliss (1934)-

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

C=growth of fungus in control.
T=growth of fungus in treatment.

Evaluation of Plant extract under in vitro condition

Nine Plant Extracts or botanicals viz. *Allium cepa, Allium sativum*, *Zingiber Officinale, Calotropic procera, Datura stramonium, Ocimum tenuiflorum, Capsicum annuum, Curcuma longa, Tagetes erecta,* were tested for their antimicrobial properties against the pathogen. One hundred grams of each plant material was collected and washed 2-3 times with water and allowed to dry at room temperature. Before extraction, each plant material (100 g) was crushed separately with 100 ml sterilized water. The plant extract was filtered through cotton cloth and centrifuged at 5000 rpm for 30 min. Then the plant extract was sterilized through autoclaving. Each plant extract was diluted in order to achieve three concentrations viz., 5, 10 and 15 per cent. Petri plates containing PDA supplemented with different plant extracts, each with three concentrations and replicated thrice were inoculated with seven days old culture (5 mm dia. disc). A control or untreated (without plant extract) was also maintained. Fungal colony was measured after 7 days of inoculation at 25 ± 1^0C. Percent over the control was calculated by the following formula suggested by Bliss (1934).

Evaluation of Bio-control under in vitro condition

For investigation of efficacy of different bioagents *viz., Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* was tested for mycelial growth inhibition of the *Sclerotinia sclerotiorum*. *In vitro,* screening of bio agents was done by dual culture technique (Dennis and Webster, 1971). Single colonies of the isolate were sub cultured in PDA and stored in refrigerator to maintain their genetic purity. Twenty ml of PDA medium was poured into sterile Petri plate and allowed for solidification. With the help of a sterile corkborer 5 mm diameter myceliadiscs from actively growing colony of culture were cut and placed near the periphery of PDA plate. Similarly, bio agents were placed on the other side *i.e.,* at an angle of 180?. Plates with no antagonists served as control for the pathogen. The plates were incubated at 25 ± 1? C for seven days. This experiment was statistically laid out with completely randomized block design with five replications. The extent of antagonistic activity by bioagent was recorded after incubation period of 7 days by measuring the growth of the test pathogen in dual culture and in control plates. Using Bliss (1934) formula the linear growth of test fungus was recorded and per cent mycelial growth inhibition was calculated.

RESULTS AND DISCUSSION

Efficacy of fungicides *in vitro*

The efficacy of nine fungicides was tested *in vitro* at three concentrations viz., 50, 100 and 150 ppm against *S. sclerotiorum* by poisoned food technique. The data (Table 1) revealed that Carbendazim, Difenconazole and Thiophenate methyl were the prime chemicals which restricted the mycelial growth of *S. sclerotiorum* at up to 100% with 50, 100 and 150 ppm concentrations followed by Captan+ Hexaconazole which inhibited 72.59%, 100 % and 100% respectively with same concentrations over untreated control. Mancozeb was found to be less effective in inhibiting the mycelial growth of *S. sclerotiorum* and exhibited 36.29 %, 48.88% and 71.48 % growth inhibition. All the treatments and their concentrations (50, 100 and 150%) were found significantly superior over the other treatments. The results of present findings in relation to Carbendazim, Difenconazole and Thiophanate methyl have a resemblance with the observations made by Shivpuri and Gupta (2001) and Pandey *et al.* (2011).

Effect of Plant Extracts on S.sclerotorium in vitro

Nine plant extracts were tested *in vitro* at three concentrations viz., 5, 10 and 15 per cent against *S. sclerotiorum* on by poisoned food technique and the data (Table 2) revealed the supremacy of garlic extract which inhibited 53.33, 66.29 and 87.77 % mycelial growth respectively with 5, 10 and 15 per cent concentration followed by Ginger extract (42.96, 53.33 and 72.59 % inhibition, respectively over control. This finding corroborated with the observations of Chattopadhyay *et al.* (2004) and Tripathi and Tripathi (2009) made against of *S. sclerotiorum.*

In-vitro assay with biocontrol agents

Results of efficacy of bio-control agent in dual culture technique (Table 2) indicated that all the tested bioagents viz., *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescence and Bacillus subtilis* were found antagonistic to the growth of *S.sclerotiorum.* Maximum 74.16 % mycelial growth inhibition of pathogen was recorded in *Pseudomonas fluorescence* followed by 61.92% in

Table 1: Efficacy of different fungicides against *Sclerotinia sclerotiorum* by poisoned food technique after 7 days of incubation at 25 + 1 0ºC

	Percent growth inhibition at different concentration(ppm)*			
Fungicide	50	100	150	Mean
Mancozeb	36.29 (37.04)	48.88 (44.35)	71.48 (57.72)	52.22 (46.27)
Carbendazim	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Carbendazim+ Mancozeb	52.07 (62.22)	73.70 (59.14)	100 (90.00)	78.64 (62.47)
Cymoxyalin + Mancozeb	47.40 (43.50)	64.81 (53.61)	81.85 (64.78)	64.69 (53.54)
Difenconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Metalaxly + Mancozeb	38.51 (38.35)	52.96 (90.00)	74.81 (59.87)	55.43 (48.11)
Captan+ Hexaconazole	72.59 (58.42)	100 (90.00)	100 (90.00)	90.86 (72.40)
Thiophenate methyl	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Chlorothanonil	64.07 (53.17)	84.81 (67.06)	100 (90.00)	82.96 (65.61)
Control	0	0	0	
S.E(d)	1.30	0.78	5.41	
C.V	2.57	1.31	7.99	
C.D 5%	2.72	1.62	11.29	

*Average of three replications
Figures given in parenthesis are angular transformed value

Table 2: Efficacy of different plant extracts against *Sclerotinia sclerotiorum* by poisoned food technique after 7 days of incubation at 25 + 1 0ºC

		Percent growth inhibition at different concentration (%) *			
Plant Extract	Part used	5%	10%	15%	Mean
Garlic	Clove	53.33 (46.91)	66.29 (54.51)	87.77 (69.53)	67.80 (55.43)
Chilli	Fruit	8.88 (17.34)	12.22 (20.46)	14.44 (22.33)	11.85 (20.14)
Onion	Blub	5.92 (14.08)	11.11 (19.46)	13.33 (21.41)	10.12 (18.55)
Turmeric	Rhizome	30.74 (33.67)	62.59 (52.29)	69.62 (56.55)	54.32 (47.48)
Tulsi	Leaves	45.18 (42.23)	50.37 (45.21)	62.96 (52.51)	52.84 (46.62)
Dartura	Leaves	22.96 (28.63)	26.66 (31.08)	37.03 (37.48)	28.88 (32.50)
Ginger	Rhizome	42.96 (40.95)	53.33 (46.90)	72.59 (58.42)	56.29 (48.61)
Akunda	Leaves	18.51 (25.48)	23.7 (29.13)	25.92 (30.61)	22.71 (28.46)
Marigold	Leaves	9.62 (18.07)	14.07 (22.03)	15.55 (23.22)	13.08 (21.81)
Control		0	0	0	
S.E(d)		1.58		1.02	
C.V		8.13		2.13	
CD 5%		2.91	3.14	3.30	

*Average of three replications
Figures given in parenthesis are angular transformed value

Table 3: *In vitro* evaluation of biological agents against growth of *S. sclerotiorum* through dual culture technique

Bio-control Agent	Mycelial growth of fungus (mm)		% Inhibition	
Trichoderma viride	40.75	(39.66)	45.27	(42.28)
Trichoderma harzianum	55.75	(48.30)	61.92	(61.92)
Pseudomonas fluorescens	66.75	(54.78)	74.16	(59.44)
Bacillus subtilis	39.25	(38.79)	42.49	(40.68)
C.V	16.18		12.72	
S.Ed	4.50		5.04	
C.D(0.5%)	9.81		10.97	

*Average of three replications; Figures given in parenthesis are angular transformed value

T. harzianum, and minimum mycelial growth inhibition was recorded in *Bacillus subtilis* (42.49 %). Similar trends of mycelial growth inhibition of pathogen was also noticed by Savchuk and Fernando (2004) with *T. harzianum, ;*Srinivasan *et al.* (2001) with *T.viride* and Shivpuri and Mali (2009) with *Bacillus.*

CONCLUSION

The present study concluded that, Carbendazim, Difenoconazole and Thiophanate methyl are the most effective fungicides which can inhibit the growth of mycelium of *S. sclerotiorum* up to 100 % inhibition at 50ppm concentration. However, plant extracts like garlic and clove can effectively inhibit the pathogen at 15% level of concentration. The bacterial bio-control agent *Pseudomonas fluorescens* can effectively minimize the growth of *S. sclerotiorum* (74.16%) in comparison to *Trichoderma harzianum*in which inhibited 61.92% under *in vitro* condition.

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